
REFERENCE

Sharma,S.K.; Dakshinamurti,K. Determination of vitamin B₆ vitamers and pyridoxic acid in biological samples, *J.Chromatogr.*, **1992**, 578, 45-51.

Pyrilamine

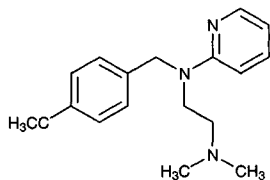
Molecular formula: C₁₇H₂₃N₃O

Molecular weight: 285.39

CAS Registry No.: 91-84-9, 59-33-6 (maleate)

Merck Index: 8168

Lednicer No.: 1 51



SAMPLE

Matrix: blood

Sample preparation: 4 mL Whole blood + 10 mL pH 10.0 phosphate buffer ($\mu = 0.4$), vortex, add 5 mL chloroform:hexane 40:60, shake gently horizontally for 30 min, centrifuge. Remove 3 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 45°, reconstitute the residue in 250 μ L dichloromethane, inject a 100 μ L aliquot.

HPLC VARIABLES

Column: 300 \times 4 10 μ m Micropak CN-10

Mobile phase: n-Hexane:dichloromethane:MeCN:pyrilamine 50:25:25:0.1

Column temperature: 30

Flow rate: 2

Injection volume: 100

Detector: UV 254

CHROMATOGRAM

Retention time: 4.0

Internal standard: pyrilamine maleate (mepyramine maleate)

OTHER SUBSTANCES

Extracted: papaverine

Simultaneous: carbetapentane, cocaine, dioxylene, ethaverine, fluphenazine, imipramine, methapyrilene, papaveraldine, procaine, promethazine, strychnine, yohimbine

Interfering: diamorphine, thonzylamine

KEY WORDS

whole blood; pyrilamine (mepyramine) is IS

REFERENCE

Hoogewijs,G.; Michotte,Y.; Lambrecht,J.; Massart,D.L. High-performance liquid chromatographic determination of papaverine in whole blood, *J.Chromatogr.*, **1981**, 226, 423-430.

SAMPLE

Matrix: formulations

Sample preparation: Crush 10 tablets, add 250 mL 50 mM HCl in EtOH:water 50:50, heat for 15 min on a steam bath, shake mechanically for 2 h, filter (glass fiber GF/A, Whatman), inject a 30 μ L aliquot of the filtrate.

HPLC VARIABLES

Column: 250 \times 4.6 10 μ m Partisil-10-ODS

Mobile phase: MeCN:buffer 50:50 (Buffer was 2.85 mM ethylenediamine sulfate adjusted to pH 7.44 \pm 0.02 with 1 M ammonium hydroxide.)

Flow rate: 3.8

Injection volume: 30

Detector: UV 216.5

CHROMATOGRAM**Retention time:** 17

OTHER SUBSTANCES**Simultaneous:** aposcopolamine, methscopolamine, pheniramine, phenylpropanolamine, tropic acid

KEY WORDStablets

REFERENCE

Heidemann, D.R. High-pressure liquid chromatographic determination of methscopolamine nitrate, phenylpropanolamine hydrochloride, pyrilamine maleate, and pheniramine maleate in tablets, *J.Pharm.Sci.*, **1981**, *70*, 820–822.

SAMPLE**Matrix:** formulations**Sample preparation:** Tablets. Powder tablets, weigh out amount equivalent to about 10 mg, add 75 mL mobile phase, sonicate for 20 min, dilute to 100 mL with mobile phase, mix, filter (0.45 μm) (discard first 10 mL of filtrate), inject a 20 μL aliquot of the filtrate. Syrups, elixirs, injectables. Measure out amount equivalent to about 10 mg, add 75 mL mobile phase, sonicate for 20 min, dilute to 100 mL with mobile phase, mix, inject a 20 μL aliquot.

HPLC VARIABLES**Column:** 300 \times 3.9 10 μm μ Bondapak CN**Mobile phase:** MeOH:3 mM ammonium acetate 90:10**Flow rate:** 1.3**Injection volume:** 20**Detector:** UV 254

CHROMATOGRAM**Retention time:** 5.0

OTHER SUBSTANCES**Also analyzed:** chlorpheniramine, cyclizine, doxylamine, mesoridazine, pentazocine, promethazine, protriptyline, pyrimethamine, tripeleennamine

KEY WORDStablets; syrups; elixirs; injections

REFERENCE

Walker, S.T. Liquid chromatographic determination of organic nitrogenous bases in dosage forms: a progress report, *J.Assoc.Off.Anal.Chem.*, **1985**, *68*, 539–542.

SAMPLE**Matrix:** formulations**Sample preparation:** Tablets. One tablet + 50 mL MeOH, sonicate, make up to 100 mL with MeOH, centrifuge for 15 min. Remove 1 mL supernatant, make up to 10 mL with mobile phase, inject a 50 μL aliquot. Drops. Dilute drops with the mobile phase so that the concentration of pyrilamine maleate is 25 $\mu\text{g/mL}$, inject a 50 μL aliquot.

HPLC VARIABLES**Column:** 100 \times 4.6 Cyclobond I (Advanced Separation Technologies)**Mobile phase:** MeOH:50 mM NaH_2PO_4 adjusted to pH 7.0 with 0.1 M NaOH 30:70**Column temperature:** 35**Flow rate:** 1.5**Injection volume:** 50**Detector:** UV 254

CHROMATOGRAM**Retention time:** 8

OTHER SUBSTANCES

Simultaneous: pheniramine, phenylpropanolamine

KEY WORDS

tablets; drops

REFERENCE

el-Gizawy,S.M.; Ahmed,A. High-performance liquid chromatographic determination of mepyramine maleate, pheniramine maleate and phenylpropanolamine hydrochloride in tablets and drops, *Analyst*, **1987**, *112*, 867-869.

SAMPLE

Matrix: formulations

Sample preparation: Crush tablets, add 100 mL water and 30-40 mL MeCN, dissolve, add N,N-dimethylbenzylamine, make up to 250 or 500 mL with water, centrifuge an aliquot, inject a 20 μ L aliquot of the supernatant.

HPLC VARIABLES

Column: 150 \times 4.6 Asahipak ODP-50 C18

Mobile phase: MeCN:200 mM pH 7.0 phosphate buffer 27:73

Flow rate: 0.8

Injection volume: 20-100

Detector: Chemiluminescence following post-column reaction. Oxidize a 1 mM tris(2,2'-bipyridine) ruthenium(II) hexachloride solution in 50 mM pH 5.5 acetate buffer to Ru(III) using a Princeton Applied Research polarographic analyzer with a platinum gauze working electrode, platinum wire auxiliary electrode, and a silver wire reference electrode, +950 mV. Pump the reagent solution at 0.28 mL/min and mix with the column effluent, allow to flow through detector. The chemiluminescence detector was a fluorescence detector with the light source removed.

CHROMATOGRAM

Retention time: 10

Internal standard: N,N-dimethylbenzylamine

OTHER SUBSTANCES

Simultaneous: brompheniramine, diphenhydramine, chlorpheniramine, pheniramine

KEY WORDS

tablets

REFERENCE

Holeman,J.A.; Danielson,N.D. Liquid chromatography of antihistamines using post-column tris(2,2'-bipyridine) ruthenium(III) chemiluminescence detection, *J.Chromatogr.A*, **1994**, *679*, 277-284.

SAMPLE

Matrix: fungal incubations

Sample preparation: Centrifuge 30 mL fungal incubation, wash pellet with water then MeOH. Combine the supernatant and the washes and add 40 mL 1 M K_2HPO_4 , extract five times with 100 mL portions of dichloromethane, filter the extracts through a plug of anhydrous sodium sulfate, evaporate the filtrate to dryness under reduced pressure at 40°, reconstitute with mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m Ultrasphere cyano

Mobile phase: MeCN:buffer 40:60 (Buffer was 10 mM KH_2PO_4 containing 20 mM trimethylamine, adjusted to pH 7.0 with aqueous KOH.)

Flow rate: 2

Injection volume: 20

Detector: UV 254

CHROMATOGRAM**Retention time:** 15

OTHER SUBSTANCES**Extracted:** metabolites

REFERENCE

Hansen, E.B., Jr.; Cerniglia, C.E.; Korfmacher, W.A.; Miller, D.W.; Heflich, R.H. Microbial transformation of the antihistamine pyrilamine maleate. Formation of potential mammalian metabolites, *Drug Metab. Dispos.*, **1987**, *15*, 97-106.

SAMPLE**Matrix:** solutions**Sample preparation:** Prepare a solution in mobile phase, inject 75-100 μ L aliquot.

HPLC VARIABLES**Column:** 250 \times 4.6 5 μ m Supelco**Mobile phase:** EtOH:MeCN:t-butylamine 98:2:0.05 (Prepared from 1 gal EtOH + 77 mL MeCN + 1.9 mL t-butylamine.)**Flow rate:** 2**Injection volume:** 75-100**Detector:** UV 254

CHROMATOGRAM**Retention time:** 2.9**Internal standard:** promazine (5.2)

OTHER SUBSTANCES

Simultaneous: N-acetylprocainamide, amitriptyline, buprion, chlordiazepoxide, chlorimipramine, chlorpheniramine, chlorpromazine, cocaine, codeine, demoxepam, desipramine, desmethylchlordiazepoxide, desmethyldisopyramide, desmethyldoxepin, dextropropoxyphene, diazepam, fluphenazine, hydroxyamoxapine (7- and 8-), 2-hydroxydesipramine, 10-hydroxynortriptyline, iminostilbene, imipramine, loxepin, maprotiline, meperidine, methadone, mianserin, morphine, nortriptyline, norzimeldine, oxapam, oxaprotiline, perphenazine, phentermine, procainamide, prochlorperazine, propoxyphene, protriptyline, quinidine, thioridazine, trifluoperazine, triflupromazine, trimeprazine, trimipramine, zimeldine

Noninterfering: thiopropazine**Interfering:** amoxapine, amphetamine, disopyramide, doxepin, 2-hydroxyimipramine, iprindole, prolixin, promethazine

KEY WORDS

normal phase

REFERENCE

Beierle, F.A.; Hubbard, R.W. Liquid chromatographic separation of antidepressant drugs: I. Tricyclics, *Ther. Drug Monit.*, **1983**, *5*, 279-292.

SAMPLE**Matrix:** solutions**Sample preparation:** Prepare a 10 μ g/mL solution in MeOH, inject a 20 μ L aliquot.

HPLC VARIABLES**Column:** 125 \times 4.9 Spherisorb S5W silica**Mobile phase:** MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7**Flow rate:** 2**Injection volume:** 20**Detector:** E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM**Retention time:** 4.3

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipiprone, diprenorphine, dipyrindamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserine, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamlamine, meclorphenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, pimindine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thiopropazine, thioridazine, thiothixene, thonzylamine, timolol, tocanide, tolpropamine, tolycaine, tranlycypromine, trazodone, trifluoperazine, trifluoperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripeleminamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane, I.; McKinnon, A.; Flanagan, R. J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J. Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 305 × 7 PRP-1 (Hamilton)

Mobile phase: Gradient. A was water:triethylamine 99.9:0.1. B was MeCN:triethylamine 99.9:0.1. A:B 60:40 for 7 min, to 20:80 over 5 min, maintain at 20:80 for 5 min, to 60:40 over 6 min, re-equilibrate at 60:40 for 2 min.

Column temperature: 40

Flow rate: 3.5

Injection volume: 500

Detector: UV 254

CHROMATOGRAM

Retention time: 16.0

OTHER SUBSTANCES

Simultaneous: diphenylpyraline, doxylamine, guaifenesin, hydrocodone, phenylephrine, phenylpropanolamine, pheniramine

Interfering: etafedrine

REFERENCE

Black,D.B.; By,A.W.; Lodge,B.A. Isolation and identification of hydrocodone in narcotic cough syrups by high-performance liquid chromatography with infrared spectrometric identification, *J.Chromatogr.*, **1986**, 358, 438-443.

SAMPLE

Matrix: solutions

Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 µg/mL, inject a 10 µL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 µm µBondapak C18

Mobile phase: MeOH:acetic acid:triethylamine:water 50:1.5:0.5:48

Flow rate: 1.5

Injection volume: 10

Detector: UV

CHROMATOGRAM

Retention time: k' 1.60

REFERENCE

Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, 370, 403-418.

SAMPLE

Matrix: solutions

Sample preparation: Prepare a solution in mobile phase, inject a 50 µL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 µm LC-CN (Supelco)

Mobile phase: MeCN:50 mM pH 7 ammonium acetate 30:70

Flow rate: 1.2-2

Injection volume: 50

Detector: E, Bioanalytical Systems LC-4B, TL-5 glassy carbon electrode, +0.9 V or UV 254

CHROMATOGRAM

Retention time: 9

OTHER SUBSTANCES

Simultaneous: pyriline N-oxide

REFERENCE

Billedeau,S.M.; Holder,C.L.; Getek,T.A. High-performance liquid chromatography of the antihistamine pyriline and its N-oxide using electrochemical detection, *J.Chromatogr.*, **1990**, 534, 151-159.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Guard column: Supelguard (Supelco)

Column: 150 × 4.6 5 µm Supelcosil LC-8-DB

Mobile phase: MeCN:MeOH:buffer 19:28:53 (Buffer was 50 mM KH₂PO₄ containing 0.2% triethylamine, pH 2.5.)

Flow rate: 1.5

Injection volume: 10

Detector: UV 254

CHROMATOGRAM

Retention time: 2

OTHER SUBSTANCES

Simultaneous: chlorcyclizine, chlorpheniramine, clonidine, diphenhydramine, promethazine, triprolidine

REFERENCE

Supelco Catalog, 1994, p. 768.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX

Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.

Column temperature: 30

Flow rate: 2

Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbitol, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepam, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlor-diazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiaacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarboxtyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephentyoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyl dopa, methyl dopamine, methylphenidate, methylprednisolone, methyltestosterone, methypylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nyldrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, pyrilalidone, quazepam, quinaldic acid, quinidine, quinine, ranitidine, rescinamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sulfadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxazole, sulfanilamide, sulfapyridine, sulfasoxazole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranlycypromine, triaminolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripeleminamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 × 4.6 12 μm 1-myristoyl-2-[(13-carboxyl)-tridecoyl]-sn-3-glycerophosphocholine chemically bonded to silica (Regis)

Mobile phase: MeCN:100 mM pH 7.0 phosphate buffer 20:80

Flow rate: 1

Detector: UV 254

CHROMATOGRAM

Retention time: k' 8.61

OTHER SUBSTANCES

Also analyzed: acebutolol, alprenolol, antazoline, atenolol, betaxolol, bisoprolol, bopindolol, bupranolol, carteolol, celiprolol, chloropyramine, chlorpheniramine, cicloprolol, cimetidine, cinarizine, cirazoline, clonidine, dilevalol, dimethindene, diphenhydramine, doxazosin, esmolol, famotidine, isothipendyl, ketotifen, metiamide, metoprolol, moxonidine, nadolol, naphazoline, nifenalol, nizatidine, oxprenolol, pheniramine, phentolamine, pindolol, pizotyline (pizotifen), practolol, prazosin, promethazine, propranolol, ranitidine, roxatidine, sotalol, tiamenidine, timolol, tramazoline, tripeleppamine, triprolidine, tymazoline, UK-14,304

REFERENCE

Kaliszan,R.; Nasal,A.; Turowski,M. Binding site for basic drugs on α₁-acid glycoprotein as revealed by chemometric analysis of biochromatographic data, *Biomed.Chromatogr.*, **1995**, *9*, 211–215.

SAMPLE

Matrix: solutions

Sample preparation: Inject a 20 μL aliquot of a 100–500 μg/mL solution in mobile phase.

HPLC VARIABLES

Column: 100 × 4.6 5 μm Hypersil C8 MOS 100A coated with phosphatidylcholine (95% pure soybean lecithin, Epikuron, Lucas Meyer & Co.) (Coat column by recycling a 1 mM solution of phosphatidylcholine in MeOH:water 80:20 for 24 h.)

Mobile phase: MeCN:35 mM pH 7.4 sodium phosphate buffer 40:60

Flow rate: 0.5–2

Injection volume: 20

Detector: UV 254

CHROMATOGRAM

Retention time: k' 2.19

OTHER SUBSTANCES

Also analyzed: amoxicillin, antipyrine, carbamazepine, chlorpheniramine, chlorpromazine, clonidine, codeine, desipramine, diphenhydramine, dipyrindamole, ephedrine, flufenamic acid, haloperidol, hydroxyzine, imipramine, indomethacin, lidocaine, megestrol acetate, metoprolol, nabumetone, nadolol, phenobarbital, phenol, promazine, propranolol, quinidine, ropinirole, testosterone, thioridazine, tolfenamic acid, verapamil

Noninterfering: acetaminophen, aspirin, azathioprine, caffeine, carprofen, chlorambucil, cimetidine, fenoterol, flurbiprofen, ibuprofen, ketoprofen, ranitidine, salicylic acid, sulfamethoxazole, theophylline, thioguanine, tiaprofenic acid, trimethoprim, valproic acid

KEY WORDS

comparison with capillary electrophoresis

REFERENCE

Hanna,M.; de Biasi,V.; Bond,B.; Salter,C.; Hutt,A.J.; Camilleri,P. Estimation of the partitioning characteristics of drugs: A comparison of a large and diverse drug series utilizing chromatographic and electrophoretic methodology, *Anal.Chem.*, **1998**, 70, 2092–2099.

Pyrimethamine

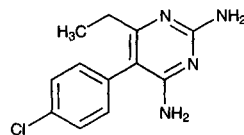
Molecular formula: C₁₂H₁₃ClN₄

Molecular weight: 248.71

CAS Registry No.: 58-14-0

Merck Index: 8169

Lednicer No.: 1 262



SAMPLE

Matrix: amniotic fluid, blood, tissue

Sample preparation: Homogenize (Ultraturrax) tissue with four volumes of physiological saline, centrifuge at 1000 g for 20 min. 200 µL Serum, tissue supernatant, or amniotic fluid + 200 µL MeCN, vortex, centrifuge at 4000 g for 10 min, inject a 50 µL aliquot of the supernatant.

HPLC VARIABLES

Column: 250 × 4.6 5 µm Spherisorb 5 ODS

Mobile phase: MeCN:water 50 :50 containing 1 g/L 85% phosphoric acid and 0.3 g/L tetra-methylammonium chloride

Flow rate: 1.5

Injection volume: 50

Detector: UV 220

CHROMATOGRAM

Limit of quantitation: 50 ng/mL

KEY WORDS

serum; monkey; pharmacokinetics; placenta; brain; heart; liver; spleen; lung

REFERENCE

Schoondermark-Van de Ven,E.; Galama,J.; Vree,T.; Camps,W.; Baars,I.; Eskes,T.; Meuwissen,J.; Melchers,W. Study of treatment of congenital *Toxoplasma gondii* infection in rhesus monkeys with pyrimethamine and sulfadiazine, *Antimicrob.Agents Chemother.*, **1995**, 39, 137–144.

SAMPLE

Matrix: blood

Sample preparation: Condition a 3 mL Bond Elut C8 SPE cartridge with 3 mL MeOH and 3 mL 50 mM pH 3.4 oxalate buffer. 1 mL Plasma + 1 mL 50 mM pH 3.4 oxalate buffer + 50 µL 200 µg/mL IS in MeOH:water 50:50, mix, add to the SPE cartridge. Wash with 3 mL 50 mM pH 3.4 oxalate buffer, 1 mL MeOH:water 20:80, and 2 mL hexane:ether 80:20. Elute with two 1 mL portions of MeOH:25% ammonia solution 99:1. Evaporate the eluate to dryness under a gentle stream of nitrogen at 30°. Dissolve the residue in 250 µL mobile phase, inject a 50 µL aliquot.

HPLC VARIABLES

Guard column: 20 × 3.9 5 µm Symmetry C18 (Waters)

Column: 250 × 4.6 5 µm Symmetry C18 (Waters)

Mobile phase: MeCN:MeOH:water 25:10:65 containing 1% triethylamine, adjusted to pH 5.6 with phosphoric acid

Column temperature: 30

Flow rate: 0.8

Injection volume: 50

Detector: UV 240

CHROMATOGRAM**Internal standard:** sulfadimethoxine**Limit of detection:** 7.01 ng/mL**Limit of quantitation:** 9.56 ng/mL

OTHER SUBSTANCES**Extracted:** sulfadoxine**Simultaneous:** acetaminophen, 4-chlorophenylbiguanide, cycloguanil, proguanil, quinine, sulfadiazine

KEY WORDSplasma; SPE

REFERENCE

Astier,H.; Renard,C.; Cheminel,V.; Soares,O.; Mounier,C.; Peyron,F.; Chaulet,J.F. Simultaneous determination of pyrimethamine and sulphadoxine in human plasma by high-performance liquid chromatography after automated liquid-solid extraction, *J.Chromatogr.B*, **1997**, 698, 217–223.

SAMPLE**Matrix:** blood

Sample preparation: Add 150 μ L 100 mM zinc sulfate to 600 μ L plasma while vortexing over 15 s, add 700 μ L MeCN containing 4 μ M WR 184806 and 75 μ M sulfadimethoxine while vortexing over 15 s, let stand for 15 min, centrifuge at 10000 g for 10 min. Remove the supernatant and add it to 2 mL pH 9.0 phosphate buffer, add 2 mL 60 mM tetrabutylammonium hydroxide, add 5 mL MTBE, shake for 10 min, centrifuge at 1200 g for 5 min. Remove the upper organic layer and evaporate it to dryness at 50°, reconstitute the residue in 200 μ L mobile phase, inject a 100 μ L aliquot.

HPLC VARIABLES**Column:** 150 \times 4 3 μ m Spherisorb S3-ODS-1**Mobile phase:** MeCN:100 mM phosphate buffer 48:52, adjusted to pH 3.5**Flow rate:** 0.5**Injection volume:** 100**Detector:** UV 229

CHROMATOGRAM**Retention time:** 8.4**Internal standard:** sulfadimethoxine (6.22), 2,8-bis(trifluoromethyl)-4-[1-hydroxy-3-(N-tert-butylamino)propyl]quinoline phosphate (WR 184806) (Walter Reed) (21.00)**Limit of detection:** 50 nM

OTHER SUBSTANCES**Extracted:** mefloquine, sulfadoxine

KEY WORDSplasma

REFERENCE

Bergqvist,Y.; Eckerbom,S.; Larsson,H.; Malekzadeh,M. Reversed-phase liquid chromatographic method for the simultaneous determination of the antimalarial drugs sulfadoxine, pyrimethamine, mefloquine and its major carboxylic metabolite in plasma, *J.Chromatogr.*, **1991**, 571, 169–177.

SAMPLE**Matrix:** blood

Sample preparation: 150 μ L Whole blood or plasma + 25 μ L monopropionyl dapson in EtOH, mix at 2200 vibrations/min for 10 min, add 100 μ L 2 M NaOH, add 3 mL MTBE, shake mechanically for 15 min, centrifuge at 1200 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 100 μ L mobile phase, vortex for 20 s, centrifuge at 1200 g for 2 min, inject an 80 μ L aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 µm Supelcosil LC-ABZ

Mobile phase: MeCN:MeOH:25 mM pH 2.3 phosphate buffer 20:10:70

Flow rate: 1.2

Injection volume: 80

Detector: UV 286

CHROMATOGRAM

Retention time: 2.5

Internal standard: monopropionyl dapsone (7.5) (Reflux dapsone with propionic anhydride in ethyl acetate for 10 min, purify by preparative TLC.)

OTHER SUBSTANCES

Extracted: monoacetyldapsone, dapsone

Noninterfering: chloroquine, quinine, sulfamethoxazole, trimethoprim, acetaminophen

Interfering: proguanil

KEY WORDS

whole blood; plasma

REFERENCE

Lemnge, M.M.; Ronn, A.; Flachs, H.; Bygbjerg, I.C. Simultaneous determination of dapsone, monoacetyldapsone and pyrimethamine in whole blood and plasma by high-performance liquid chromatography, *J. Chromatogr.*, **1993**, 613, 340–346.

SAMPLE

Matrix: blood

Sample preparation: Whole blood. 150 µL Whole blood + 25 µL 800 ng/mL monopropionyl dapsone + 1.2 mL 200 mM NaOH + 5 mL MTBE, mix for 25 min, centrifuge at 1600 g for 10 min. Remove 4 mL of the organic layer and evaporate it to dryness under a stream of air at 37°, reconstitute the residue in 100 µL mobile phase, inject an 80 µL aliquot. Dried blood. Let 150 µL blood dry on filter paper. Cut paper into small pieces, add 25 µL 800 ng/mL monopropionyl dapsone, add 1.2 mL 200 mM NaOH, mix gently for 30 min, add 5 mL MTBE, mix for 25 min, centrifuge at 1600 g for 10 min. Remove 4 mL of the organic layer and evaporate it to dryness under a stream of air at 37°, reconstitute the residue in 100 µL mobile phase, inject an 80 µL aliquot.

HPLC VARIABLES

Guard column: 20 × 4.6 Supelguard LC-ABZ (Supelco)

Column: 150 × 4.6 5 µm Supelcosil LC-ABZ

Mobile phase: MeCN:MeOH:buffer 14:7:49 (Buffer was 25 mM phosphate adjusted to pH 2.3 with orthophosphoric acid.)

Flow rate: 1.2

Injection volume: 80

Detector: UV 286

CHROMATOGRAM

Retention time: 2.3

Internal standard: monopropionyl dapsone (7.1)

Limit of quantitation: 20 ng/mL

OTHER SUBSTANCES

Extracted: monoacetyldapsone, metabolites, dapsone

Noninterfering: acetaminophen, chloroquine, quinine, sulfadoxine, sulfamethoxazole, trimethoprim

KEY WORDS

whole blood; dried blood

REFERENCE

Ronn,A.M.; Lemnge,M.M.; Angelo,H.R.; Bygbjerg,I.C. High-performance liquid chromatography determination of dapson, monoacetyldapson, and pyrimethamine in filter paper blood spots, *Ther.Drug Monit.*, **1995**, *17*, 79–83.

SAMPLE

Matrix: blood

Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 µL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 µL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 µm NovaPack C18

Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH₂PO₄ adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)

Column temperature: 30

Flow rate: 0.8

Injection volume: 50

Detector: UV 273

CHROMATOGRAM

Retention time: 5.00

Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur—compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoyllecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; tolaxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyrindamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phenacyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzone; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; aceprometazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrrodine; phenylbutazone; demexiptiline; clozapine; progualil; trifluoperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozone; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tiocloamarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; caripramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254-262.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 µm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30

Detector: UV 208.7

CHROMATOGRAM

Retention time: 12.497

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149-163.

SAMPLE

Matrix: formulations

Sample preparation: Tablets. Powder tablets, weigh out amount equivalent to about 10 mg, add 75 mL mobile phase, sonicate for 20 min, dilute to 100 mL with mobile phase, mix, filter (0.45 µm) (discard first 10 mL of filtrate), inject a 20 µL aliquot of the filtrate. Syrups, elixirs, injectables. Measure out amount equivalent to about 10 mg, add 75 mL mobile phase, sonicate for 20 min, dilute to 100 mL with mobile phase, mix, inject a 20 µL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 µm µBondapak CN

Mobile phase: MeOH:3 mM ammonium acetate 90:10

Flow rate: 1.3

Injection volume: 20

Detector: UV 254

CHROMATOGRAM

Retention time: 2.7

OTHER SUBSTANCES

Also analyzed: chlorpheniramine, cyclizine, doxylamine, mesoridazine, pentazocine, promethazine, protriptyline, pyrilamine, tripeleminamine

KEY WORDS

tablets; syrups; elixirs; injections

REFERENCE

Walker, S.T. Liquid chromatographic determination of organic nitrogenous bases in dosage forms: a progress report, *J. Assoc. Off. Anal. Chem.*, **1985**, *68*, 539–542.

SAMPLE

Matrix: formulations

Sample preparation: Powder tablets, add 40 mg pyrimethamine, dissolve in 20 mL MeCN, add 40 mL mobile phase, filter (paper), wash filter with mobile phase, make up filtrate to 100 mL with mobile phase. Dilute a 5 mL aliquot to 50 mL with mobile phase, inject a 20 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4 10 μ m Nucleosil C18

Mobile phase: MeOH:MeCN:water:triethylamine 55:5:40:0.1, pH adjusted to 4.0 with phosphoric acid

Flow rate: 1.5

Injection volume: 20

Detector: UV 254

CHROMATOGRAM

Retention time: 3.5

Internal standard: pyrimethamine

OTHER SUBSTANCES

Simultaneous: aspirin, dipyridamole

KEY WORDS

tablets; pyrimethamine is IS

REFERENCE

Sane, R.T.; Ghadge, J.K.; Jani, A.B.; Vaidya, A.J.; Kotwal, S.S. Simultaneous high-performance liquid chromatographic determination of haloperidol with propantheline bromide, nalidixic acid with phenazopyridine hydrochloride, and dipyridamole with aspirin in combined dosage (forms), *Indian Drugs*, **1992**, *29*, 240–244.

SAMPLE

Matrix: solutions

Sample preparation: Prepare a 10 μ g/mL solution in MeOH, inject a 20 μ L aliquot.

HPLC VARIABLES

Column: 125 \times 4.9 Spherisorb S5W silica

Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7

Flow rate: 2

Injection volume: 20

Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 1.7

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzocetamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazepine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipiphenone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, er-

gosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserine, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamlamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylegonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranlycypromine, trazodone, trifluoperazine, trifluoperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripeleennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane, I.; McKinnon, A.; Flanagan, R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J. Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

Sample preparation: Prepare a solution in mobile phase, inject a 25 μL aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 5 μm Zorbax-Sil

Mobile phase: Dichloromethane:MeOH:1 M perchloric acid 100:9:0.4

Flow rate: 0.8

Injection volume: 25

Detector: UV 254

CHROMATOGRAM

Retention time: 9.6

OTHER SUBSTANCES

Simultaneous: amodiaquine, chloroquine, dapson, desethylchloroquine, dihydroquinine, mefloquine, primaquine, proguanil, quinidine, quinine, sulfadoxine, sulfalene, sulfamethoxazole

Interfering: dihydroquinidine

KEY WORDS

normal phase

REFERENCE

Dua, V.K.; Sarin, R.; Prakash, A. Determination of quinine in serum, plasma, red blood cells and whole blood in healthy and *Plasmodium falciparum* malaria cases by high-performance liquid chromatography, *J. Chromatogr.*, **1993**, *614*, 87–93.

SAMPLE

Matrix: solutions

Sample preparation: Prepare a solution in mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 300 \times 3.9 10 μm $\mu\text{Bondapak C18}$

Mobile phase: MeCN:MeOH:1 M perchloric acid:water 30:9:0.8:95

Flow rate: 1.5

Injection volume: 20

Detector: UV 254

CHROMATOGRAM

Retention time: k' 7.70

OTHER SUBSTANCES

Simultaneous: amodiaquine, chloroquine, dapson, primaquine, quinidine, quinine, sulfadoxine, sulfalene, sulfamethoxazole

REFERENCE

Dua,V.K.; Sarin,R.; Sharma,V.P. Sulphadoxine concentrations in plasma, red blood cells and whole blood in healthy and *Plasmodium falciparum* malaria cases after treatment with Fansidar using high-performance liquid chromatography, *J.Pharm.Biomed.Anal.*, **1994**, 12, 1317–1323.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 300 × 0.35 5 µm Vydac IDI-TP C18 TMS capped

Mobile phase: Gradient. MeCN:MeOH:buffer 0:0:100 at start of run, to 0:5:95 after injection (step gradient), to 0:8:92 over 7 min, to 6:0:94 (step gradient), maintain at 6:0:94 for 14 min, to 0:16:84 over 5 min, to 0:18:82 over 5 min, to 0:30:70 over 5 min. (Buffer was 1 mM pH 2.72 phosphate buffer.)

Column temperature: 30

Flow rate: 0.006

Injection volume: 1

Detector: UV 270

CHROMATOGRAM

Retention time: 48

OTHER SUBSTANCES

Simultaneous: diaveridine, phthalyl sulfathiazole, succinyl sulfathiazole, sulfabenzamide, sulfacetamide, sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfaguanidine, sulfamerazine, sulfameter, sulfamethazine, sulfamethizole, sulfamethoxazole, sulfamethoxypyridazine, sulfamoxole, sulfanilamide, sulfanilic acid, sulfapyridine, sulfaquinoxaline, sulfathiazole, sulfisomidine, sulfisoxazole, trimethoprim

KEY WORDS

capillary HPLC

REFERENCE

Ricci,M.C.; Cross,R.F. High performance liquid chromatographic analyses of sulphonamides and dihydrofolate reductase inhibitors. II. Separations in acetonitrile modified solutions, ternary gradient studies & flow programming, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, 19, 547–564.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 300 × 0.35 5 µm Vydac IDI-TP C18 TMS capped

Mobile phase: Gradient. MeOH:buffer 0:100 at start of run, to 10:90 after injection (step gradient), to 12:88 over 30 min, to 18:82 over 5 min, to 30:70 over 5 min. (Buffer was 1 mM pH 2.72 phosphate buffer)

Column temperature: 30

Flow rate: 0.006

Injection volume: 1

Detector: UV 270

CHROMATOGRAM**Retention time:** 57

OTHER SUBSTANCES

Simultaneous: diaveridine, phthalyl sulfathiazole, succinyl sulfathiazole, sulfabenzamide, sulfacetamide, sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfaguanidine, sulfamerazine, sulfameter, sulfamethazine, sulfamethizole, sulfamethoxazole, sulfamethoxypyridazine, sulfamoxole, sulfanilamide, sulfanilic acid, sulfapyridine, sulfaquinoxaline, sulfathiazole, sulfisomidine, sulfisoxazole, trimethoprim

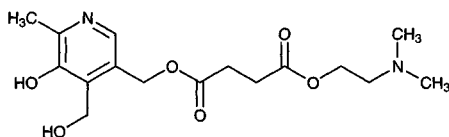
KEY WORDS

capillary HPLC

REFERENCE

Ricci, M.C.; Cross, R.F. High-performance liquid chromatographic analyses of sulphonamides and dihydrofolate reductase inhibitors. I. Separations in methanol-modified solutions, *J. Liq. Chromatogr. Rel. Technol.*, **1996**, *19*, 365–381.

Pyrisuccideanol

Molecular formula: C₁₆H₂₄N₂O₆**Molecular weight:** 340.38**CAS Registry No.:** 33605-94-6, 53659-00-0 (dimaleate)**Merck index:** 8175

SAMPLE**Matrix:** blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200–350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES**Guard column:** 20 mm long Symmetry C18**Column:** 250 × 4.6 5 µm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10–30**Detector:** UV 209.9

CHROMATOGRAM**Retention time:** 3.462

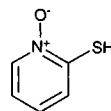
KEY WORDS

whole blood

REFERENCE

Gaillard, X.; Pépin, G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J. Chromatogr. A*, **1997**, *763*, 149–163.

Pyrithione



Molecular formula: C_5H_5NOS

Molecular weight: 127.17

CAS Registry No.: 1121-30-8, 13463-41-7 (zinc derivative)

Merck Index: 8178

SAMPLE

Matrix: formulations

Sample preparation: Weigh out shampoo containing 10 mg zinc pyrithione and make up to 100 mL with buffer saturated with chloroform (if phases separate use water saturated with chloroform), shake well for a few min, sonicate for a few min. Remove a 10 mL aliquot and add it to 10 mL chloroform saturated with water, add 2 mL 1 M copper(II) sulfate, shake vigorously for 5 min, centrifuge at 1500 g for 5 min, inject a 5 μ L aliquot of the lower organic layer. (Buffer was 100 mM citric acid:200 mM Na_2HPO_4 97:103, pH 5.0.)

HPLC VARIABLES

Column: 150 \times 4.6 5 μ m Nucleosil 5C18

Mobile phase: MeOH:water 60:40 (Before use flush system with 0.1% EDTA at 0.5 mL/min, then water.)

Column temperature: 25

Flow rate: 1

Injection volume: 5

Detector: UV 320

CHROMATOGRAM

Retention time: 15

Limit of quantitation: 100 ng

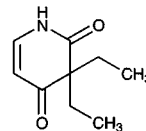
KEY WORDS

shampoo; complexation; copper complexes

REFERENCE

Nakajima,K.; Yasuda,T.; Nakazawa,H. High-performance liquid chromatographic determination of zinc pyrithione in antidandruff preparations based on copper chelate formation, *J.Chromatogr.*, **1990**, 502, 379–384.

Pyrithyldione



Molecular formula: $C_9H_{13}NO_2$

Molecular weight: 167.21

CAS Registry No.: 77-04-3

Merck Index: 8179

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 \times 4.6 Zorbax RX

Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.

Column temperature: 30

Flow rate: 2

Detector: UV 210

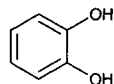
OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, am-triptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepam, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlor-diazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fen-camfamine, fenpropfen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiaicol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, imino-stilbene, imipramine, indomethacin, isocarboxtyril, isocarboxazid, isoniazid, isoproterenol, isox-suprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, metha-pyrylene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyl dopa, methyl dopamine, methylphenidate, methylprednisolone, methyl-testosterone, methylpyrrol, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nyldrin, oxazepam, oxycodone, ox-ymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendi-metrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phenter-mine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, predni-solone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recin-amine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopalamine, scopolotin, secobarbital, strychnine, sulfacetamide, sulfadiazine, sulfadimethoxine, sulfaethi-dole, sulfamerazine, sulfamethazine, sulfamethoxazole, sulfanilamide, sulfapyridine, sulfasox-azole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, the-baine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tol-metin, tranlycypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripeleonnamine, triprolidine, tropacocaine, tyramine, verapa-mil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill, D.W.; Kind, A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J. Anal. Toxicol.*, **1994**, *18*, 233–242.

Pyrocatechol



Molecular formula: C₆H₆O₂

Molecular weight: 110.11

CAS Registry No.: 120-80-9

Merck Index: 8183

SAMPLE

Matrix: solutions

Sample preparation: Aqueous food simulants. Pipette 1.0 mL 200 mg/L IS in MeOH into a 25 mL volumetric flask and dilute to the mark with the food simulant obtained from migration experiment, shake. Repeat the procedure to obtain a duplicate sample, filter a portion through a 0.2 µm membrane filter, inject a 20 µL aliquot. Olive oil simulants. Weigh 25 g olive oil food simulant obtained from migration experiment into a beaker, pour oil into a separating funnel, allow beaker to drain for 30 s. Rinse it with 25 mL hexane and add washes to separating funnel. Add 1.0 mL 200 mg/L IS in MeOH into funnel and mix. Add 10 mL water, shake vigorously by hand for 30 s, allow to stand for 5 min. Collect aqueous phase and reextract oil with a 10 mL water. Combine aqueous extracts, make up to 25 mL with water, filter the extracts through a small cotton plug to remove any entrained oil. Repeat the procedure to obtain a duplicate sample. Inject a 20 µL aliquot. (Aqueous food simulants were: distilled water, 3% acetic acid in water; EtOH:water 15:85.)

HPLC VARIABLES

Column: 250 × 4.6 5 µm Hypersil ODS

Mobile phase: MeCN:buffer 15:85 (Prepare mobile phase as follows. Dissolve 7.5 g sodium dihydrogen orthophosphate in 800 mL water, add 150 mL MeCN and adjust to pH 3.6 with glacial acetic acid. Make up to 1000 mL with water.)

Flow rate: 1

Injection volume: 20

Detector: UV 280

CHROMATOGRAM

Retention time: 8.7

Internal standard: 2-methyl-1,3-dihydroxybenzene (7.4)

Limit of detection: 300-400 ng/g

OTHER SUBSTANCES

Extracted: hydroquinone, resorcinol

KEY WORDS

aqueous food simulants; olive oil simulants

REFERENCE

Philo, M.R.; Jickells, S.M.; Castle, L. Testing for compliance with migration limits: Determination of 1,2-, 1,3-, and 1,4-dihydroxybenzenes in food-simulating solvents by liquid chromatography, *JAOAC Int.*, **1996**, 79, 746-750.

Quazepam

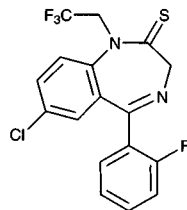
Molecular formula: C₁₇H₁₁ClF₄N₂S

Molecular weight: 386.80

CAS Registry No.: 36735-22-5

Merck Index: 8211

Lednicer No.: 3 196



SAMPLE

Matrix: blood

Sample preparation: Condition a Bond Elut C8 SPE cartridge with 2 mL MeOH and 2 mL water, do not allow to dry. Add 100 µL 1 µg/mL diazepam in 1 M pH 10.5 glycine buffer then 500 µL plasma to the column, wash with 2 mL water, wash with 50 µL MeOH, elute with three 200 µL portions of MeOH, combine the eluates and evaporate them to dryness under a stream of nitrogen at 37°, reconstitute the residue in 100 µL mobile phase, inject a 50-80 µL aliquot.

HPLC VARIABLES

Column: 75 × 3.9 Nova-Pak C18

Mobile phase: MeOH:2 mM pH 7.2 phosphate buffer 60:40